

This note describes a simultaneous determination method for 9 phenolic antioxidants using a binary gradient HPLC system.

Among them, propyl gallate (PG), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are permitted as food additive in Japan. Meanwhile, 2,4,5- trihydroxybutyrophenone (THBP), *tert*-butylhydro- quinone (TBHQ), 4-hydroxymethyl-2,6-di-*tert*-butyl-

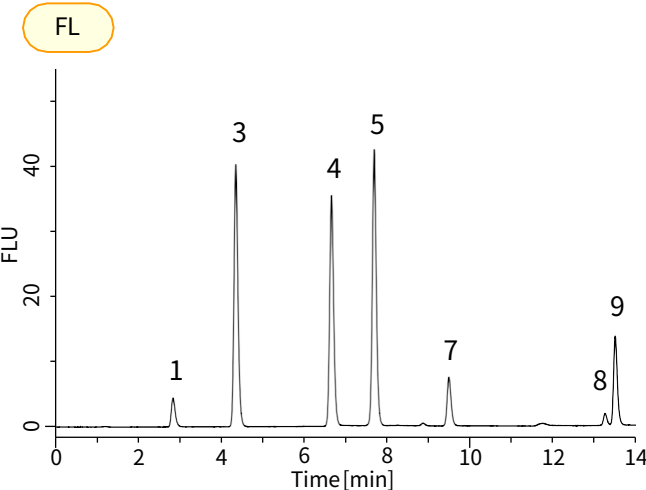
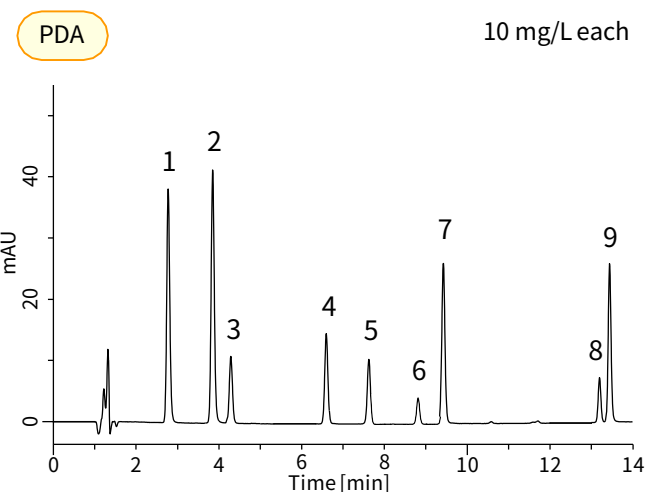
phenol (HMBP), octyl gallate (OG), and dodecyl gallate (DG) are used in other countries.

All of the phenolic antioxidants can be determined with a photodiode array (PDA) detector. Fluorescence detection

also can be utilized except for THBP and HMBP. In this note, a PDA detector and a fluorescence detector were connected in series, and the chromatograms obtained with both detectors were shown.

(K.Suzuki)

Chromatograms obtained from standard solution



1. Propyl gallate (PG)
2. 2,4,5-Trihydroxybutyrophenone (THBP)
3. *tert*-Butylhydroquinone (TBHQ)
4. Nordihydroguaiaretic acid (NDGA)
5. Butylated Hydroxyanisole (BHA)
6. 4-Hydroxymethyl-2,6-di-*tert*-butylphenol (HMBP)
7. Octyl gallate (OG)
8. Butylated hydroxytoluene (BHT)
9. Dodecyl gallate (DG)

HPLC Conditions

Column : Inertsil ODS-SP
(5 μ m, 150 x 4.6 mm I.D.)
Cat.No. 5020-02745

Eluent : A) CH₃OH
B) CH₃CN
C) 5% Acetic acid
A/B/C = 20/20/60 - 15 min
- 50/50/0
(equilibration 10 min) , v/v/v
(Mixed by a gradient mixer)

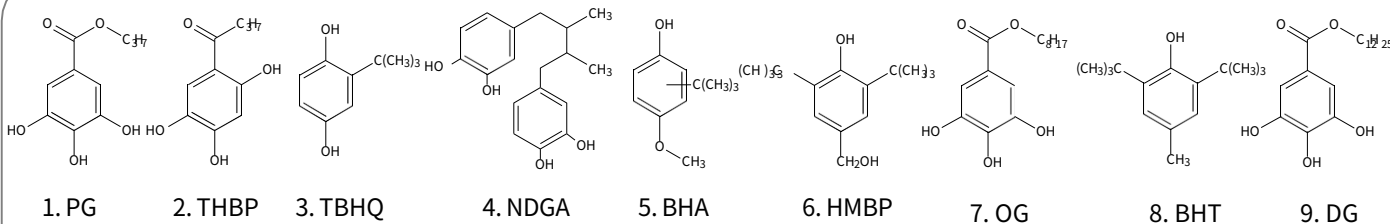
Flow rate : 1.5 mL/min

Col. Temp. : 40 °C

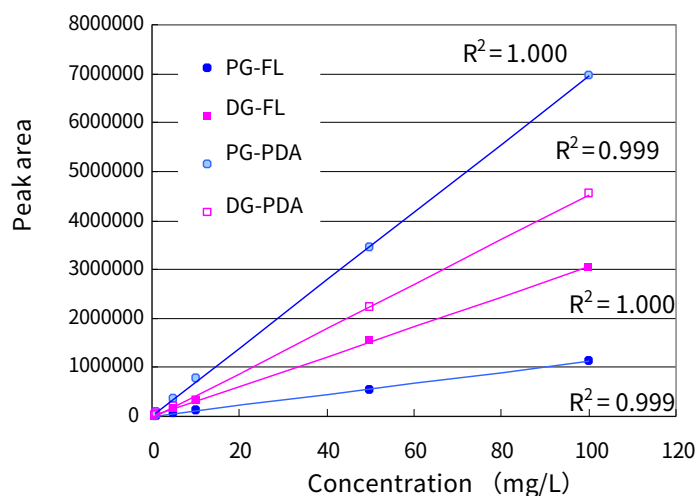
Detection : PDA 280 nm
FL Ex. 275 nm, Em. 365 nm

Inj. Volume : 10 μ L

Chemical Structures



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovayon Software, Inc.



		R ²	
		PDA	FL
1. PG	:	1.000	0.999
2. THBP	:	1.000	—
3. BHQ	:	1.000	0.997
4. NDGA	:	1.000	0.998
5. BHA	:	1.000	0.999
6. HMBP	:	1.000	—
7. OG	:	1.000	0.999
8. BHT	:	0.998	0.999
9. DG	:	0.999	1.000

The calibration curves and their correlation coefficients

An example of sample pretreatment

Chromatograms obtained from food sample (margarine)

Sample

5 g

Extraction

Sodium sulfate (anhydrous) 5 g
 $\text{CH}_3\text{CN}/\text{IPA}/\text{C}_2\text{H}_5\text{OH}=2/1/1$ 50 mL
 Homogenize for 2 min
 Cool at -5°C for 1 hr
 Centrifuge at 3000 rpm for 10 min

Supernatant

Residue
 Rinse with 15 mL of
 $\text{CH}_3\text{CN}/\text{IPA}/\text{C}_2\text{H}_5\text{OH}$
 $=2/1/1$
 — Centrifuge at 3000 rpm
 for 10 min

Supernatant

Residue

Waste

Concentration

Evaporate to < 2 mL at temperature lower
 than 40°C

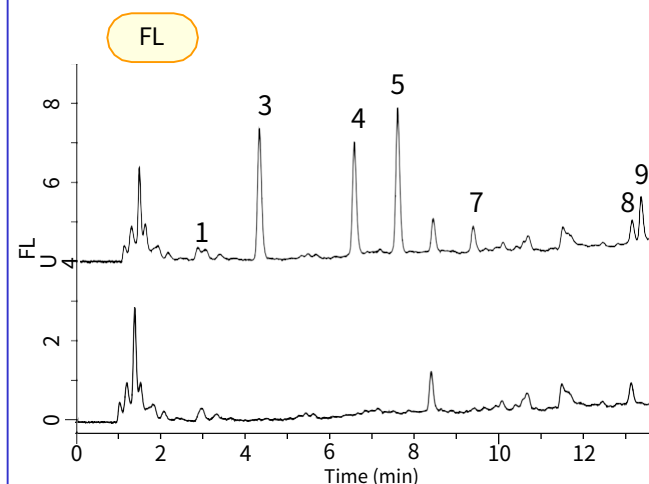
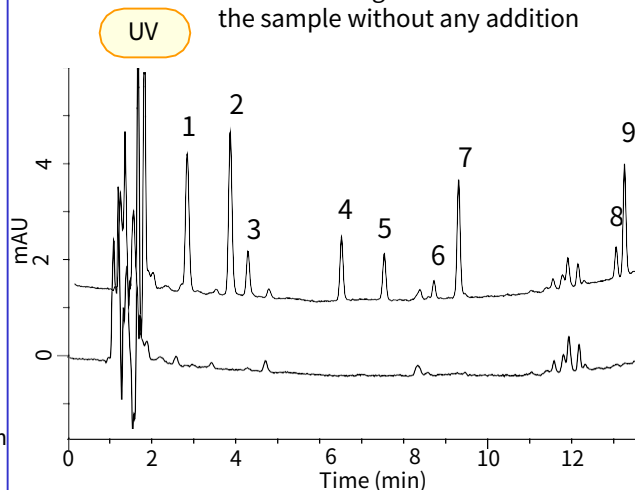
Preparation for injection

Make up to 5 mL with $\text{CH}_3\text{CN}/\text{IPA}/\text{C}_2\text{H}_5\text{OH}$
 $=2/1/1$
 Filtrate with $0.45\mu\text{m}$ membrane filter

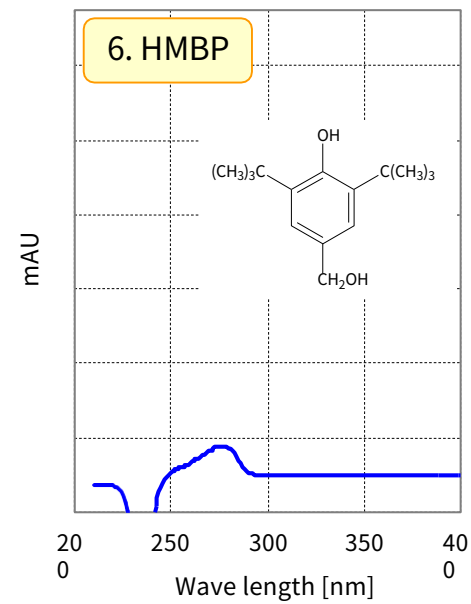
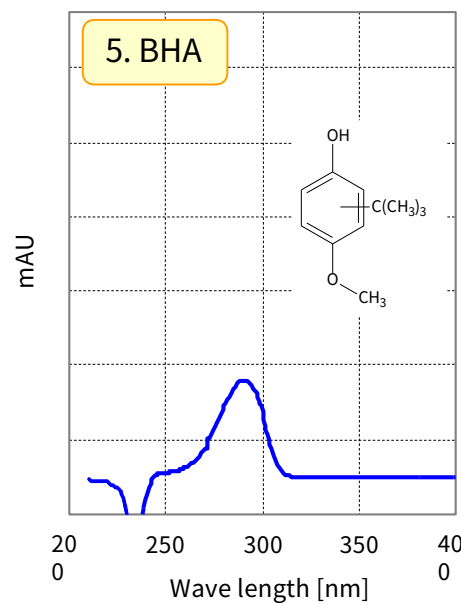
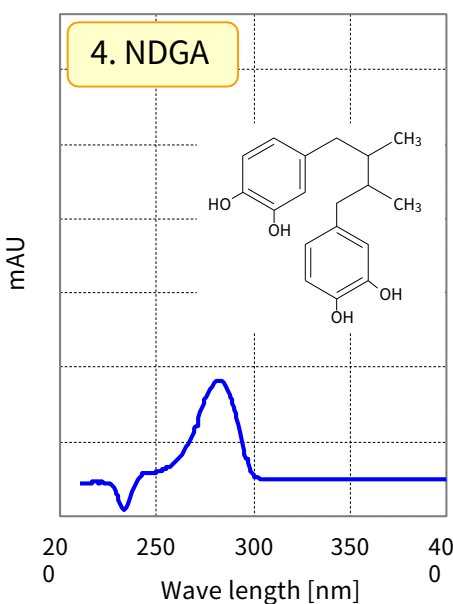
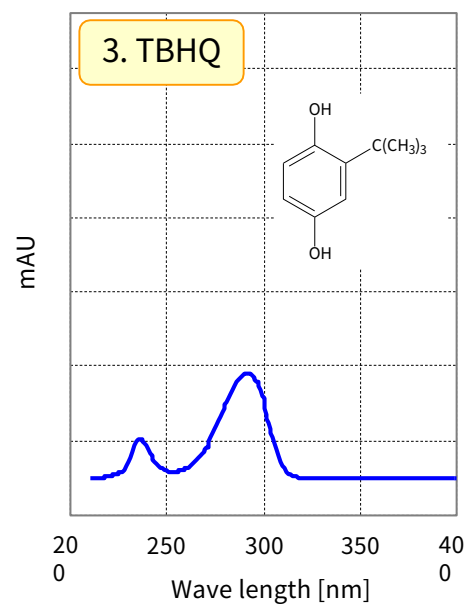
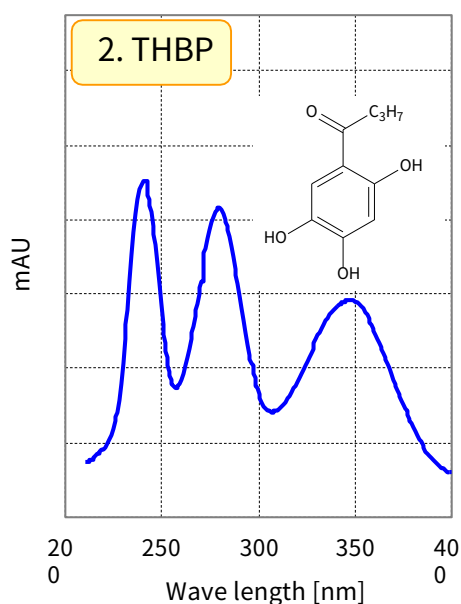
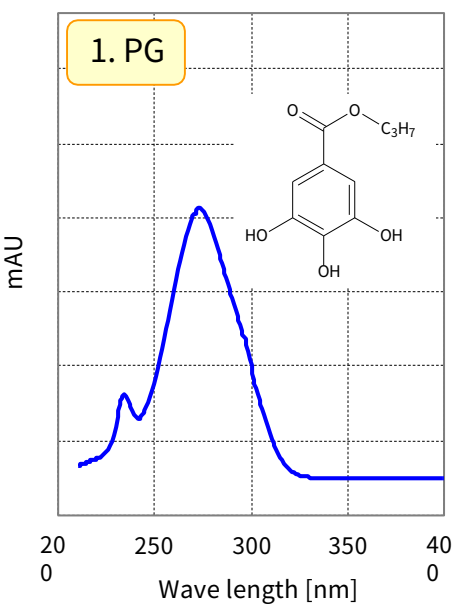
HPLC

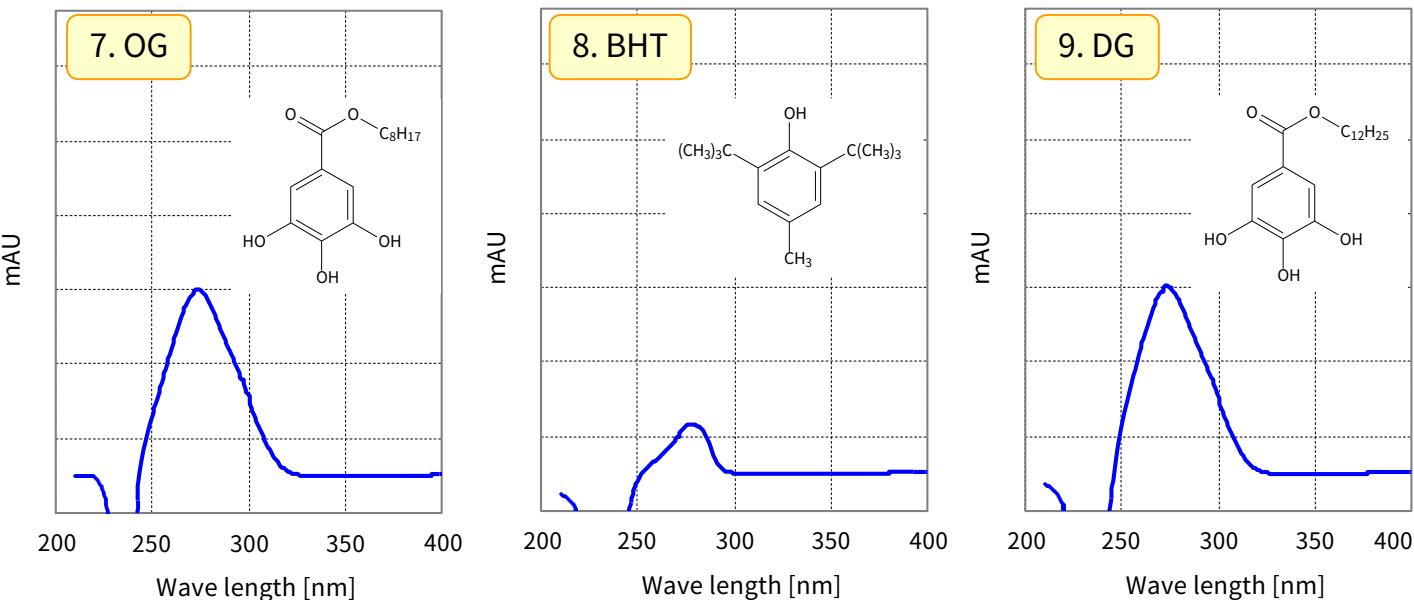
Upper chromatogram:
 the sample with standard addition (1.0 mg/L
 each)

Lower chromatogram:
 the sample without any addition



The adsorption spectra obtained with PDA detector





* The absorbance scale of each spectrum was all the same.

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GL Sciences Inc. Japan

22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@glsc.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA

4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.

Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited

Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn



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